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# Preparation and Characterization of Antisera to Electrophoretically Purified SA11 Virus Polypeptides

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Antisera to SA11 virus proteins were prepared by immunizing rabbits with individual polypeptides separated by polyacrylamide gel electrophoresis under reducing or nonreducing conditions; the resulting antisera were characterized by four immunological methods. Results of complement fixation tests with double-shelled rotavirus particles and sera raised against reduced or unreduced proteins of the outer shell of the virus suggested the presence of common antigenic determinants in the outer capsid layers of SA11 and the Northern Ireland strain of calf rotavirus. In this test, antisera to outer shell polypeptides gp34 ($O_2$) and gp25 ($O_4$) cross-reacted with calf rotavirions, whereas those to p62 ($O_1$) and p26 ($O_3$) reacted only with the homologous virus. Antisera to the reduced outer shell proteins of the virus did not neutralize viral infectivity, nor did they possess hemagglutination inhibition activity. Evidence suggesting the presence of type-specific antigenic determinant(s) in the major inner protein p42 ($I_4$) of SA11 virus, capable of inducing neutralizing antibody, is presented and discussed. Antisera produced against unreduced gp34 and p26 polypeptides of the virus contained type-specific neutralizing antibodies. Polypeptide gp34 was also capable of inducing hemagglutination inhibiting antibody. All of the antisera to unreduced polypeptides had agglutinating activity against double-shelled particles of homologous and heterologous rotaviruses.

The simian rotavirus SA11 (strain $H_{96}$) was isolated from a rectal swab taken from a healthy vervet monkey (19). The morphology of the virus has been described previously (6) and noted as typically rotaviral in nature (12). Morphologically, rotaviruses resemble reovirus; two types of particles have been observed in preparations from all of the animal species in which they have been found. Single-shelled particles consist of an inner capsid of 60-nm diameter, and double-shelled particles have also an outer capsid of 70-nm diameter (12, 30). The double-shelled virions of SA11 virus consist of at least eight structural polypeptides (25). There are five polypeptides in the inner capsid ranging in molecular weight from 42,000 to 113,000, and three or four polypeptides are detected in the outer shell with a molecular weight range of 25,000 to 62,000.

All known rotaviruses share a common antigen which has been associated with the inner capsid layer (3, 14, 27, 30). With further studies differences associated with the outer capsid layer of the virions began to show up, and now they are distinguishable from each other (although there is some cross-reaction) by serum neutralization (10, 27, 30), complement fixation (32), immunoelectron microscopy (3), enzyme-linked immunosorbent assay (31, 33), and hemagglutination inhibition (8). Thus, it appears that there are group- and type-specific antigens associated with the rotavirus particle which elicit the production of antibodies that are detectable by in vitro tests. Therefore, there are several bases for assuming that sera raised against single polypeptide chains will be useful in defining the antigenic characteristics of rotaviruses; what is more, monospecific antibodies of this kind could provide the information necessary to establish the functions of the individual rotavirion components. Recently, Flewett et al. (9) and Beards et al. (2) suggested that there may well be more than one polypeptide reacting in the neutralization reaction with rotaviruses. In this paper, we support this conclusion and report differences in the reactivity of antisera raised against reduced or unreduced SA11 virus polypeptides separated by polyacrylamide gel electrophoresis (PAGE).

## MATERIALS AND METHODS

**Cells.** MA-104 cells, an established line derived from rhesus monkey kidneys, were obtained from S. Matsuno of the National Institute of Health of Japan,

Tokyo. Cells were cultivated with Eagle minimal essential medium supplemented with 10% fetal calf serum (Commonwealth Serum Laboratories, Melbourne, Australia) and containing gentamicin and fungizone. CV-1 cells, an established line derived from African green monkey kidneys, were kindly supplied by CSIRO Division of Animal Health, Melbourne, Australia. These cells were cultured in medium 199 (GIBCO), containing 10% fetal calf serum, gentamicin, and amphotericin B.

**Viruses.** The simian rotavirus SA11 was received from H. Malherbe, San Antonio, Tex., and the Northern Ireland strain of calf (NIC) rotavirus was kindly supplied by M. S. McNulty, Belfast, Northern Ireland. Both the SA11 and NIC rotaviruses were grown in MA-104 cells. After virus adsorption for 1 h at 37°C, minimal essential maintenance medium containing 10 μg of trypsin (Difco Laboratories, Detroit, Mich.; 1:250) per ml, gentamicin, and amphotericin B was added to the bottles. Infected monolayers were incubated at 37°C and were harvested when they showed extensive cytopathic effects. The method described by Rodger et al. (24) with minor modifications (1) was followed for the purification of SA11 virus. The same procedure was used for purification of NIC rotavirus, but 10 mM calcium chloride was added to the solutions of sucrose, cesium chloride, and sodium chloride to stabilize the double-shelled particles during purification.

**Preparation of single-shelled particles of rotaviruses.** Based on the observation of Cohen (4) that treatment of rotavirus with ethylenediaminetetraacetic acid (EDTA) at 37°C causes a definite shift of the virus buoyant density from 1.36 to 1.38 g/ml, the following procedure was used for the preparation of SS particles of rotaviruses. Purified virions suspended in 0.002 M tris(hydroxymethyl)aminomethane (Tris)-hydrochloride (pH 7.5) were treated with 1 mM EDTA at 37°C for 30 min. After incubation, the EDTA-treated virus was sedimented through a 35% (wt/wt) sucrose cushion at 85,000 × *g* for 1 h in a Spinco SW65 rotor. The virus pellet was suspended in 0.5 ml of 0.002 M Tris buffer and then layered on 5 ml of a 36% (wt/wt) cesium chloride solution in 0.2 M Tris buffer. After centrifugation at 179,000 × *g* for 18 h at 4°C in a Spinco SW65 rotor, the band of single-shelled particles was harvested and dialyzed overnight against 0.14 M NaCl.

**Sodium dodecyl sulfate-PAGE.** Purified virus particles were heated at 100°C for 2 min in Laemmli sample buffer, and the polypeptides were separated on 8.75% polyacrylamide vertical slab gels by the Tris-glycine buffer system of Laemmli (16). Separations of viral polypeptides under nonreducing conditions were also carried out. In this case the reducing agent 2-mercaptoethanol was omitted during the preparation of the sample buffer. The nomenclature adopted for polypeptides is based on molecular weight estimates as suggested previously (25), but the estimates are our most recent (5). The corresponding Thouless (26) designations are included in parenthesis.

**Polypeptide extraction for CF tests.** After electrophoresis, individual viral polypeptides were extracted from polyacrylamide gels as described by Lane and Robbins (17). The eluted polypeptides were dialyzed overnight at 4°C against 0.14 M saline before their use as antigens in complement fixation (CF) titrations.

**Preparation of gel fragments for immunization.** After PAGE of SA11 virus, the gel was cut into vertical strips. One strip was stained overnight with 0.05% Coomassie brilliant blue in methanol-acetic acid-water (1:1:1) followed by destaining in 7.5% acetic acid, and the remainder of the strips were wrapped in Saran Wrap and stored at 4°C. The stained strip was kept in the destaining solution until it reached the same length as the unstained sections. The stained bands were used to locate the polypeptide bands in the unstained gels, and then the bands from 10 strips were excised with a scalpel blade. Techniques for immediate visualization of proteins (11, 20) were not satisfactory since the minor protein bands were not visible. The excised gel blocks (usually from 10 strips) containing each protein band were extruded twice through a 5-ml glass syringe. The material was then homogenized in 2 ml of 0.14 M saline in a tissue grinder (Arthur H. Thomas Co., Philadelphia, Pa.) and sonicated for 30 s (Measuring & Scientific Equipment Ltd., London).

**Antisera to reduced polypeptides.** For each rabbit, 2 ml of the gel processed as above was mixed with an equal volume of Freund adjuvant. Emulsions were prepared and injected in multiple subcutaneous sites along the back of outbred New Zealand rabbits, according to the following immunization schedule. The first injection was in complete adjuvant, followed 2 weeks later by a second injection in incomplete adjuvant. After a period of 3 weeks, a third injection in incomplete adjuvant was administered. Animals were bled 7 and 14 days later. One month after the third injection, a booster immunization was given in incomplete adjuvant. Animals were again bled as described above. Sera were stored at −70°C.

**Antisera to unreduced polypeptides.** Antisera to unreduced polypeptides were raised by subcutaneous and intramuscular injections of 2 ml of homogenized gel preparations emulsified in equal volumes of Freund adjuvant (complete for the first injection and incomplete for subsequent injections). The rabbits were given three immunizations at weekly intervals, and then a booster immunization was administered 3 weeks later. At 1 and 2 weeks after the final injection rabbits were bled, and serum samples were stored at −70°C.

**CF test.** The CF test employed was the microtitration method of the U.S. Public Health Service (28). Briefly, it is a method using a 2.8% suspension of sheep erythrocytes, 1 optimal sensitizing dose of hemolytic serum, 1 optimal dilution of test antigen, and 5 50% hemolytic doses of complement (stabilized, from guinea pig; Commonwealth Serum Laboratories). Antigen, serum, and CF diluent controls were performed with 5, 2.5, and 1.25 U of complement. The diluent used throughout consisted of Oxoid CF test diluent tablets dissolved as recommended (Oxoid Ltd., London). Disposable polystyrene plates (U-bottomed; Sterilin Limited, Teddington, England) were used.

**Hemagglutination inhibition test.** The hemagglutination inhibition test was performed with microtiter equipment as described by Fauvel et al. (8). Before hemagglutination inhibition tests sera were treated with kaolin and adsorbed with packed human

group O erythrocytes. Antigen controls (back titration of the 4HAU of the virus) were included in each test.

**Neutralization test.** Neutralization of SA11 virus was assayed on either MA-104 or CV-1 cells, since the efficiency of plaque formation was identical in both cell lines. Neutralization of NIC rotavirus was assayed only in CV-1 cells since this virus did not plaque in MA-104 cells by the procedure used. Serial dilutions of antisera were prepared in virus diluent (Hanks balanced salts solution containing 0.2% gelatin, essential for virus stability during the reaction), and then an equal volume of virus containing about 100 plaque-forming units per 0.1 ml was added. After 60 min at room temperature cell monolayers were rinsed with phosphate-buffered saline (pH 7.2) and inoculated with the virus-serum mixture. After 1 h of adsorption, cells were overlaid with minimal essential medium-0.5% agarose containing antibiotics and 10 μg of trypsin per ml by the method of Matsuno et al. (21). After 3 days of incubation at 37°C, cells were fixed and stained as described by Ramia and Sattar (22). Neutralizing titers were expressed as reciprocals of the highest serum dilution causing a 50% reduction in plaque counts compared with the corresponding preimmune serum.

**Immunoelectron microscopy.** The agar gel diffusion filtration method essentially as described by Kelen et al. (15) was followed for immunoelectron microscopy. Volumes of 10 μl of a suspension of SA11 or NIC rotavirus particles were added to equal volumes of 1:10 dilutions of antisera. The mixtures were incubated for 1 h at 37°C and then overnight at 4°C; 5 μl of each virus-serum mixture was deposited on the surface of a microscope slide that had previously been covered with 0.8% agar, and then a Formvar-coated specimen grid was placed upside down and left floating on top of the drop. When diffusion was complete, the grids were removed for negative staining with one-tenth-saturated ammonium molybdate and examined with a Hitachi HU 11A electron microscope. Criteria similar to those described by Bridger (3) were followed for counting the virus particles for each virus-serum mixture and for calculating the number and size of aggregates. Clumps of more than three particles were taken to indicate that agglutination had occurred.

## RESULTS

**Preparation of antisera to reduced or nonreduced polypeptides of SA11 virus separated by PAGE.** The structural proteins of SA11 virus purified on cesium chloride gradients were separated by PAGE as described above. The reducing agent 2-mercaptoethanol was used at a concentration of 5% in the sample buffer for separation of reduced polypeptides, but was omitted when nonreduced polypeptides were required. The proteins strongly stained by Coomassie blue were clearly visible on the gel strip (Fig. 1A) and were subsequently used to locate the viral polypeptides in the unstained gel strips. The optimum gel concentration was found to be 8.75%, as such gels permitted easy control of size variations on staining and destaining and were easily emulsified for injection. The polypeptide bands were only slightly affected by diffusion during storage overnight at 4°C, shown by some decrease in the intensity of the staining, but the positions of the different polypeptide bands remained unchanged (Fig. 1*b*).

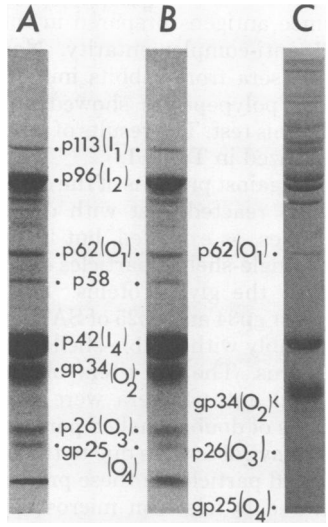


FIG. 1. *Isolation of inner (I) and outer (O) shell polypeptides of SA11 virus by PAGE under reducing (A and B) or nonreducing conditions (C). The polypeptide bands used as immunogens were located by staining one strip of the gel with Coomassie blue immediately after electrophoresis (A) and then excised. A strip stained after storage overnight at 4°C (B) showed little diffusion of the bands occurred during the time required for destaining of the first strip. Separation was in 8.75% polyacrylamide slab gels, and migration was from top to bottom.*

The distribution of nonreduced polypeptides in marker gel is shown in Fig. 1C. Note the increased mobilities of gp34, p26, and gp25 suggesting intramolecular disulfide cross-linking. The high-molecular-weight bands are mainly aggregates of p42 (I. Lazdins, M. Dyall-Smith, and I. Holmes, manuscript in preparation). Polypeptide gp25 was not always seen in gels run under nonreducing conditions, so the immunization schedule with this protein consisted of only two injections. Rabbits received four injections of the other polypeptides, as described above.

**CF titrations.** All rabbit antipolypeptide sera, except anti-$I_1$ serum, reacted in CF tests with either double-shelled or single-shelled rotavirus particles. The potential problem of the presence of complete virus particles in preparations of single-shelled particles obtained by density gradient centrifugation in cesium chloride was avoided by the use of EDTA as described above. Attempts to perform CF tests with SA11 virus polypeptides extracted from polyacrylamide gels after electrophoresis proved unsuc-

cessful since antigens prepared in this manner exhibited anti-complementarity. None of the preimmune sera from rabbits immunized with SA11 virus polypeptides showed antirotaviral activity by this test. The results of repeated tests are summarized in Table 1.

Antisera against proteins of the outer (O) shell of the virus reacted best with double-shelled virus particles, as expected, but low-titer reactions with single-shelled particles did occur with sera against the glycoproteins. The sera prepared against gp34 and gp25 of SA11 virus cross-reacted notably with double-shelled particles of NIC rotavirus. The 1:8 titers obtained when anti-p96 and anti-p42 sera were reacted with preparations of double-shelled particles of SA11 and NIC rotaviruses were due to the presence of single-shelled particles in these preparations, as demonstrated by electron microscopy. On the whole the results indicate group-specific antigenic determinants on the inner capsid, best detected with the antiserum to p42, and suggest the presence of both type- and group-specific determinants on the outer capsid layer of SA11 rotavirus.

**Hemagglutination inhibition and neutralization titrations.** None of the outer shell polypeptides of SA11 virus prepared under reducing conditions induced hemagglutination inhibition or neutralizing antibodies. Surprisingly, the anti-p42 serum gave a reaction in neutralization with complete SA11 virus, but not with complete NIC rotavirus particles (Table 2). Mixing of this antiserum with equal volumes (50 μl) of each of the antisera raised to outer shell polypeptides did not enhance its neutralizing activity. Preimmune serum obtained from the rabbit immunized with p42 showed no neutralizing or hemagglutination-inhibiting activity.

Antisera prepared against the unreduced outer shell proteins were more promising for establishing the polypeptides responsible for infectivity and hemagglutination activities of SA11 virus. In contrast to the results obtained with reduced polypeptides, unreduced gp34, the major outer shell component of the virus, induced significant neutralizing and hemagglutination-inhibiting activity. In addition, polypeptide p26

TABLE 2. *Results of hemagglutination inhibition and neutralization titrations of antisera against reduced or nonreduced SA11 polypeptides separated by PAGE*

| Antiserum to SA11 polypeptide | Treatment of polypeptide | Reciprocal of titer by: | | |
|---|---|---|---|---|
| | | HAI[a] | NT[b] | |
| | | SA11 | SA11 | NIC |
| p62 | Reduced | <10 | <20 | ND[c] |
| p62 | Nonreduced | <20 | <25 | <25 |
| p42 | Reduced | <10 | 200 | <10 |
| gp34 | Reduced | <10 | <20 | ND |
| gp34 | Nonreduced | 160 | 1,600 | <25 |
| p26 | Reduced | <10 | <20 | ND |
| p26 | Nonreduced | <20 | 200 | <25 |
| p62, gp34, p26, gp25, p42 | Reduced | <50 | 200 | ND |
| Preimmune serum | | <10 | <20 | <10 |

[a] All sera to be used in hemagglutination inhibition (HAI) tests were treated with kaolin and adsorbed with human group O erythrocytes, so the starting dilution of each serum in the mixture was 1:50.

[b] Neutralization (NT) titers are expressed as the highest serum dilution causing a 50% reduction in plaque counts.

[c] ND, not done.

TABLE 1. *Results of CF titrations with antisera against reduced or nonreduced SA11 virus polypeptides separated by PAGE*

| Antiserum to SA11 polypeptide | Treatment of polypeptide | Antiserum titer[a] against: | | | |
|---|---|---|---|---|---|
| | | SA11 (DS)[b] | SA11 (SS)[c] | NIC (DS) | NIC (SS) |
| p113 ($I_1$) | Reduced | <8 | <8 | <8 | <8 |
| p96 ($I_2$) | Reduced | 8 | 16 | 8 | 8 |
| p62 ($O_1$) | Reduced | 16 | <8 | <8 | <8 |
| p62 ($O_1$) | Nonreduced | 8 | <8 | <8 | <8 |
| p58 | Reduced | 16 | <8 | <8 | <8 |
| p42 ($I_4$) | Reduced | 8 | 64 | 8 | 64 |
| gp34 ($O_2$) | Reduced | 32 | 8 | 16 | <8 |
| gp34 ($O_2$) | Nonreduced | 128 | 8 | 64 | 8 |
| p26 ($O_3$) | Reduced | 32 | <8 | <8 | <8 |
| p26 ($O_3$) | Nonreduced | 32 | <8 | <8 | <8 |
| gp25 ($O_4$) | Reduced | 16 | <8 | 8 | <8 |
| gp25 ($O_4$) | Nonreduced | 16 | 8 | 8 | 8 |
| Control[d] | | <8 | <8 | <8 | <8 |

[a] Expressed as the reciprocal of serum dilution which gave a reading of 30% hemolysis when titrated with an optimal dilution of test antigen.

[b] DS, Double shelled.

[c] Single-shelled (SS) particles of both SA11 and NIC rotaviruses were prepared by EDTA treatment of virions.

[d] Preimmune serum from rabbit later immunized with polypeptide p42.

induced neutralizing but not hemagglutination-inhibiting activity. The neutralization was type specific in each instance.

**Immunoelectron microscopy.** Antisera raised against inner shell proteins of SA11 rotavirus agglutinated single-shelled particles of homologous and heterologous viruses. The results for anti-p42, which demonstrated the strongest reaction, are shown in Table 3. This antiserum also had a weaker, but significant, agglutinating activity against double-shelled particles of SA11, but not against those of NIC virus.

The antisera prepared against nonreduced outer capsid polypeptides had much greater agglutinating activity than those produced with the corresponding reduced forms and also showed a greater type specificity, though there was a considerable amount of cross-reaction in this test.

## DISCUSSION

Purified SA11 virus antigens were separated by PAGE under reducing and nonreducing conditions and used to produce antisera which reacted with the native antigens on the virus particle. Results of CF titrations seem to indicate the presence of group- and type-specific antigens on the outer shell of rotavirions. Thus, antisera raised against reduced or unreduced polypeptides gp34 and gp25 of SA11 virus reacted with double-shelled particles of both SA11 and NIC rotaviruses, whereas antisera to polypeptides p62 and p26 reacted only with the homologous double-shelled particle form of the virus. These results are supported in part by those of Zissis and Lambert (32) who were able to distinguish two serotypes of human rotavirus by CF and immunoelectron microscopy. These authors established that the type-specific receptors are located at the periphery of the virions and that the different types might also share a minor surface antigen responsible for the heterologous agglutination observed.

TABLE 3. *Agglutination of single- or double-shelled particles of SA11 and NIC rotaviruses by antisera against reduced or nonreduced polypeptides of SA11*

| Antiserum to: | Polypeptide treatment | % agglutination[a] | | | |
|---|---|---|---|---|---|
| | | Single-shelled particles | | Double-shelled particles | |
| | | SA11 | NIC | SA11 | NIC |
| p62 | Nonreduced | ND | ND | 43 | 32 |
| p42 | Reduced | 91 | 93 | 39 | 0 |
| gp34 | Reduced | 9 | 11 | 11 | 0 |
| gp34 | Nonreduced | ND | ND | 100 | 50 |
| p26 | Reduced | ND | ND | 14 | 11 |
| p26 | Nonreduced | ND | ND | 100 | 45 |
| gp25 | Reduced | ND | ND | 17 | 11 |
| gp25 | Nonreduced | ND | ND | 49 | 31 |
| Control[b] | | 0 | 0 | 0 | 0 |

[a] Percentage of virus particles in aggregates of >3 particles; 200 to 300 particles were counted. ND, Not done.

[b] Preimmune rabbit sera from rabbits later immunized with p42 (reduced) or gp34 (nonreduced).

Even though hemagglutination inhibition and neutralization are two serological tests associated with the outer capsid layer of rotaviruses, none of the reduced outer shell proteins of SA11 virus induced hemagglutination-inhibiting or neutralizing antibodies. This observation suggests that the immunogenic capacity of the proteins in the outer shell of the virus which induce the production of these types of antibodies was affected by denaturation or reduction in the sodium dodecyl sulfate-polyacrylamide gels used in the preparation of the immunogens.

The most surprising finding in this study was the fact that antiserum prepared against the major inner shell protein (p42) of the virus showed a slight neutralizing activity for SA11, but not for NIC rotavirus. The test was repeated three times with the same result. Contamination of this polypeptide with the major outer glycoprotein (gp34) of the virus during preparation of the immunogen seems unlikely since the anti-gp34 (reduced) serum itself is devoid of neutralizing activity. Furthermore, combination of anti-p42 serum with equal volumes of each of the antisera directed to outer shell proteins of the virus did not enhance its neutralizing activity. A possible explanation for this observation could be that denaturation of the immunizing p42 antigen may have exposed determinants which are partially internal in nature. Therefore, the antiserum directed against the denatured polypeptide may recognize these partially hidden determinants exposed by the presence of holes or breaks in the outer shell (3, 7). Only a limited number of such sites would be accessible, which could account for the low neutralizing titer. The presence of both common and type-specific antigens on a single protein has been demonstrated for the influenza virus hemagglutinin (18, 29) and for herpes simplex virus types 1 and 2 (23).

In view of the failure to produce neutralizing and hemagglutination-inhibiting antibodies with reduced outer shell polypeptides of SA11 virus, it seemed possible that the reduction with 5% 2-mercaptoethanol may have led to confirmational changes in the polypeptide backbone, altering their immunogenicity. This assumption is supported by the recent findings of Kaluza and Pauli (13) who reported that treatment with 2-mercaptoethanol causes reductive cleavage of intramolecular disulfide bridges in the integral glycoproteins of Semliki forest virus and alters the tertiary structure of these proteins. In addition, they noted that reduction with even 2% 2-

mercaptoethanol destroys hemagglutinating activity and infectivity of the virion. Our own results (1) also support this view as pretreatment of purified SA11 virus with either *p*-hydroxymercuribenzoate, which reacts with sulfhydryl groups, or sodium sulfite, which reacts with disulfide bonds, inactivated the hemagglutinating capacity of the virus. It was consequently decided to separate viral immunogens in the absence of 2-mercaptoethanol. Antisera produced against immunogens prepared in this manner provided the first evidence that two substructures, gp34 and p26, of the virus can induce production of type-specific neutralizing antibodies in rabbits. Furthermore, polypeptide gp34 was also capable of inducing the production of hemagglutination-inhibiting antibody. The lack of neutralizing activity in the antiserum against polypeptide gp25 does not seem to be due to the smaller amount of protein used as immunogen, since both agglutinating and CF antibodies were induced. Polypeptides p26 and gp25 are closely related and may differ only in degree of glycosylation (M. Dyall-Smith, personal communication).

Bridger (3) noted that double-shelled particles of calf rotavirus were only agglutinated by anti-human rotavirus serum when the particular serum possessed neutralizing activity to that calf strain. They were not agglutinated by anti-human or anti-pig rotavirus sera that had no such neutralizing activity. However, our results of neutralization and agglutination tests with monospecific antisera to unreduced polypeptides seem to indicate that any antiserum directed against antigens of the outer shell (with or without neutralizing activity) can agglutinate double-shelled particles of homologous and heterologous rotaviruses.

Results of hemagglutination inhibition tests indicated that the hemagglutinating activity of SA11 virus resides in an antigenic determinant(s), present on the glycoprotein gp34, which represents the major outer shell component of the virus. Even though it has been suggested that the carbohydrate moiety of SA11 virus glycoproteins is not directly involved in the hemagglutinating capacity of the virus (1), further immunochemical approaches are needed to define the determinants more precisely.

The ability to prepare antisera to polypeptides which contain the antigenic determinants responsible for the hemagglutination activity and infectivity of SA11 rotavirus could be very useful in studies of rotavirus serotypes and may assist the interpretation of epidemiological investigations based on gel electrophoresis of viral ribonucleic acid; it could represent a new beginning of our understanding of this virus.

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## LITERATURE CITED

1. **Bastardo, J. W., and I. H. Holmes.** 1980. Attachment of SA11 rotavirus to erythrocyte receptors. Infect. Immun. **29**:1134–1140.
2. **Beards, G. M., J. N. Pilfold, M. E. Thouless, and T. H. Flewett.** 1980. Rotavirus serotypes by serum neutralization. J. Med. Virol. **5**:231–237.
3. **Bridger, J. C.** 1978. Location of type-specific antigens in calf rotaviruses. J. Clin. Microbiol. **8**:625–628.
4. **Cohen, J.** 1977. Ribonucleic acid polymerase activity associated with purified calf rotavirus. J. Gen. Virol. **36**: 395–402.
5. **Dyall-Smith, M. L., and I. H. Holmes.** 1981. Gene-coding assignments of rotavirus double-stranded RNA segments 10 and 11. J. Virol. **38**:1099–1103.
6. **Els, H. J., and G. Lecatsas.** 1972. Morphological studies on simian virus SA 11 and the "related" O agent. J. Gen. Virol. **17**:129–132.
7. **Esparza, J., and F. Gil.** 1978. A study on the ultrastructure of human rotavirus. Virology **91**:141–150.
8. **Fauvel, M., L. Spence, L. A. Babiuk, R. Petro, and S. Bloch.** 1978. Hemagglutination and hemagglutination-inhibition studies with a strain of Nebraska calf diarrhea virus (bovine rotavirus). Intervirology **9**:95–105.
9. **Flewett, T. H., J. A. Shirley, and G. M. Beards.** 1979. Rotavirus epidemiology a biochemical approach. INSERM **90**:53–62.
10. **Flewett, T. H., M. E. Thouless, J. N. Pilfold, A. S. Bryden, and J. A. N. Candeias.** 1978. More serotypes of human rotavirus. Lancet **ii**:632.
11. **Hartman, B. K., and S. Udenfriend.** 1969. A method for immediate visualization of proteins in acrylamide gels and its use for preparation of antibodies to enzymes. Anal. Biochem. **30**:391–394.
12. **Holmes, I. H., B. J. Ruck, R. F. Bishop, and G. P. Davidson.** 1975. Infantile enteritis virus: morphogenesis and morphology. J. Virol. **16**:937–943.
13. **Kaluza, G., and G. Pauli.** 1980. The influence of intramolecular disulfide bonds on the structure and function of Semliki forest virus membrane glycoproteins. Virology **102**:300–309.
14. **Kapikian, A. Z., W. L. Cline, H. W. Kim, A. R. Kalica, R. G. Wyatt, D. H. Van Kirk, R. M. Chanock, D. H. James, and A. L. Vaughn.** 1976. Antigenic relationships among five reovirus-like (RVL) agents by complement fixation (CF) and development of new substitute CF antigens for the human RVL agent of infantile gastroenteritis. Proc. Soc. Exp. Biol. Med. **152**:535–539.
15. **Kelen, A. E., A. E. Hathaway, and D. A. McLeod.** 1971. Rapid detection of Australia/SH antigen and antibody by a simple and sensitive technique of immunoelectron microscopy. Can. J. Microbiol. **17**:993–1000.
16. **Laemmli, U. K.** 1970. Cleavage of structural proteins during the assembly of the head of bacteriophage T4. Nature (London) **227**:680–685.
17. **Lane, D. P., and A. K. Robbins.** 1978. An immunochemical investigation of SV40 T antigens. I. Production properties and specificity of a rabbit antibody to purified simian virus 40 large-T antigen. Virology **87**:182–193.
18. **Laver, W. G., and J. C. Downie.** 1974. Studies on antigenic variation in influenza virus. Evidence for multiple antigenic determinants on the hemagglutinin subunits of A/Hong Kong/68(H3N2) virus and the A/England/72 strains. Virology **59**:230–244.

19. **Malherbe, H. H., and M. Strickland-Cholmley.** 1967. Simian virus SA 11 and the related O agent. Arch. Gesamte Virusforsch. **22**:235–245.
20. **Malik, N., and A. Berrie.** 1972. New stain fixative for proteins separated by gel isoelectric focusing based on Coomassie brilliant blue. Anal. Biochem. **49**:173–176.
21. **Matsuno, S., S. Inouye, and R. Kono.** 1977. Plaque assay of neonatal calf diarrhea virus and the neutralizing antibody in human sera. J. Clin. Microbiol. **5**:1–4.
22. **Ramia, S., and S. A. Sattar.** 1979. Simian rotavirus SA 11 plaque formation in the presence of trypsin. J. Clin. Microbiol. **10**:609–614.
23. **Randall, R. E., R. A. Killington, and D. H. Watson.** 1980. Glycoproteins with type common and type specific antigenic sites excreted from cells infected with herpes simplex virus types 1 and 2. J. Gen. Virol. **48**:297–310.
24. **Rodger, S. M., R. D. Schnagl, and I. H. Holmes.** 1975. Biochemical and biophysical characteristics of diarrhea viruses of human and calf origin. J. Virol. **16**:1229–1235.
25. **Rodger, S. M., R. D. Schnagl, and I. H. Holmes.** 1977. Further biochemical characterization, including the detection of surface glycoproteins, of human, calf, and simian rotaviruses. J. Virol. **24**:91–98.
26. **Thouless, M. E.** 1979. Rotavirus polypeptides. J. Gen. Virol. **44**:187–198.
27. **Thouless, M. E., A. S. Bryden, T. H. Flewett, G. N. Woode, J. C. Bridger, D. R. Snodgrass, and J. A. Herring.** 1977. Serological relationship between rotaviruses from different species as studied by complement fixation and neutralization. Arch. Virol. **53**:287–294.
28. **U.S. Public Health Service.** 1965. Standardized diagnostic complement fixation method and adaptation to micro test. Public Health Monograph no. 74. U.S. Public Health Service Publication no. 1228. Government Printing Office, Washington, D.C.
29. **Virelizier, J. L., A. C. Allison, and G. C. Schild.** 1974. Antibody responses to antigenic determinants of influenza virus hemagglutinin. II. Original antigenic sin: a bone marrow-derived lymphocyte memory phenomenon modulated by thymus-derived lymphocytes. J. Exp. Med. **140**:1571–1578.
30. **Woode, G. N., J. C. Bridger, J. M. Jones, T. H. Flewett, A. S. Bryden, H. A. Davies, and G. B. B. White.** 1976. Morphological and antigenic relationships between viruses (rotaviruses) from acute gastroenteritis of children, calves, piglets, mice and foals. Infect. Immun. **14**:804–810.
31. **Yolken, R. H., R. G. Wyatt, G. Zissis, C. D. Brandt, W. J. Rodriguez, H. W. Kim, R. H. Parrott, J. J. Urritia, L. Mata, H. B. Greenberg, A. Z. Kapikian, and R. M. Chanock.** 1978. Epidemiology of human rotavirus types 1 and 2 studied by enzyme-linked immunosorbent assay. N. Engl. J. Med. **299**:1156–1161.
32. **Zissis, G., and J. P. Lambert.** 1978. Different serotypes of human rotaviruses. Lancet **i**:38–39.
33. **Zissis, G., and J. P. Lambert.** 1980. Enzyme-linked immunosorbent assays adapted for serotyping of human rotavirus strains. J. Clin. Microbiol. **11**:1–5.